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Systematics and nesting biology of large carpenter bee, *Xylocopa* (*Biluna*) *nasalis* Westwood, 1838 (Hymenoptera: Apidae: Xylocopinae) from India

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Abstract
Though the large carpenter bees are well known pollinators of cultivated and native plants, there is an information gap regarding wild bees in India. The present study is a contribution to the knowledge of the systematics and nesting architecture of *Xylocopa (Biluna) nasalis* Westwood, 1838 from India. Males and females of *X. nasalis* species are diagnosed, described, and illustrated, including details of the male genitalia. Taxonomic comments, data on the geographical distribution and morphological measurements are given in detail to aid their identification by pollination researchers and notes on nesting biology are documented. The study was conducted during January 2016 in Chinthamani near Bengaluru. Thirteen nests were collected for dissection for measurements. Nests of *X. nasalis* were unbranched and constructed in pithy or hollow stem. The mass provisioned cells were separated by a diaphragm. The nest entrances were located at the middle of the bamboo culms. The average nest length was 29.50±1.84 cm with a mean internal diameter of 1.44±0.25 cm. The number of cells per nest ranged from 2 to 8 ($\bar{x}$ = 5.17±2.14). Detailed nest architecture is provided in this paper which may help in designing artificial trap nests for conserving these pollinators.

Keywords: Pollinators, wild bees, biodiversity, conservation, bamboo nesting, xylocopini

Introduction
The subfamily Xylocopinae includes two superficially very different kinds of bees - species of the tribe Xylocopini are large to very large, robust euceriform to anthophoriform bees, whereas the other three tribes, Allodapini, Ceratinini and Manueliini are represented by small, slender, andreniform to almost hylaeiform bees [1]. The Xylocopini consists of a single genus, *Xylocopa* Latreille, which are commonly known as large carpenter bees because nearly all species build their nests in burrows in dead wood, bamboo, or structural timbers, except those in the subgenus *Proxylocopa*, which nests on the ground [2]. Members of the related tribe Ceratinini are sometimes referred to as small carpenter bees. Large carpenter bees are conspicuous, commonly encountered, and fascinating elements of the melittofauna in many regions of the world [3]. There are about 500 species of *Xylocopa* in 31 subgenera are known in the word [1]. With their large body size, behaviour of floral constancy and buzzing, these are considered as important pollinators of several cultivated and wild species of plants [4]. There are two main types of nests among the woodnesting *Xylocopa* species: one is unbranched or linear nests in which the tunnel runs in the same direction as the nest entrance or at most with a single right angle corner from the nest entrance and second is branched nests which consist of at least two or more tunnels, although with only one nest entrance [5]. Species of the subgenus *Biluna* Maa [6], are only known to construct unbranched nests in bamboo culms [7, 8, 2] and its distribution ranges from India and Sri Lanka to southeast Asia and Japan [9]. *Xylocopa* (*Biluna*) *nasalis* Westwood [10], is a species commonly found throughout Southeast Asia. It superficially resembles the sympatric species *X.* (*Mesotrichia*) *latipes* Drury [11] and *X.* (*M.*) *tenuiscapa* Westwood [12], because of the presence of black pubescence on the mesosoma and size (21–35 cm in length) [9]. The behavior, biology and natural history of *X. nasalis*, is poorly known even though it is commonly found throughout rural and agricultural areas. The present study is an attempt to bridge the gap in knowledge about its nesting biology. Females are involved in nest construction, digging tunnels or holes in the selected wood with their strong and well developed mandibles, followed by a of pollen mixed with nectar from different flower types available in the habitat for transfer to brood chambers in the nesting hosts.

While it takes two months for the juvenile broods to emerge, the same nest is reused over and over again, unless it is disturbed [13, 14]. The nesting architectural details should prove to be beneficial to beekeepers and researchers who are interested in trap nesting and conservation of carpenter bees.

2. Material and Methods

Materials examined in this study are deposited in the Department of Agricultural Entomology, University of Agricultural Sciences, Gandhi Krishi Vignan Kendra, Bengaluru, India. External morphological structures were studied using a Nikon SMZ 1000 stereomicroscope and habitus photographs were taken with a Canon 7Ddigital camera with a 100mm macro lens. Multiple images at different focal depths were taken and combined using Zeren stacker software. Male genitalia dissections were carried out by relaxing the specimen and detaching last metasomal segment with a micro pin needle and then digested with 10% KOH for 24 hours. The genital capsule was carefully separated from metasomal segments and washed thoroughly with water, then the genital capsule was transferred to test tubes containing 70 per cent ethyl alcohol. The different skeletal parts were separated under a stereo-binocular microscope and placed on a cavity block with glycerol. Genitalia of each specimen was stored in small eppendorf tubes containing a few drops of glycerol for subsequent studies. Genitalia structureswere photographed using a Leica 205C microscope mounted with a Leica DFC450 camera. Plates were prepared using Adobe Photoshop CS5.The terminology used in the study is that proposed by earlier authors [6, 2, 15, 1]. All measurements are given in millimeters and were measured using a Leica M205C microscope with auto measurement and analysis system.The nesting biology of *X. nasalis* was studied in Chinthamani ($12^0$23'N, $77^0$03'E, 887m,), approximately 75 km from Bengaluru. Nests were found on 16 January 2016 in Sericulture College campus bamboo plants garden. Total of 13 nests were collected and prior to collection the nests were observed for bee activity. Nests were sealed with cello tape by closing the entrance with small stone to avoid escape of carpenter bees and were brought into the laboratory for dissection and nest architecture study. Later nests were measured and photographed using Canon 7D digital camera with a 100 mm macro lens.

3. Results

Systematics

***Xylocopa* (*Biluna*) *nasalis* Westwood, 1838**

Xylocopa dissimilis Lepeletier [16]
Xylocopa lunulata Lepeletier [16]
Xylocopa minensis Cockerell [17]
Female (Figures 1a & 1b)

Pubescence: Pubescence dominantly black, fore femora with less pubescence; outer portion of hind tibia with thick band of stout setae.

Head: Face without any pale markings; labrum with one strong tubercle and 3 or 4 weak tubercles; mandibles bidentate, outer tooth slightly longer than inner tooth, outer groove not prominent, base with triangular depression; median ocelli with crescent shaped unpunctate black band; apical end of clypeus unpunctate; face slightly depressed at the base of the antennae; inner margin of orbits slightly converging at upper extremities.

Mesonotum: Pronotum with distinct unpunctate area; propodeum not elevated like in male but with a weak median furrow and a triangular unpunctate area; posterior part of tegulae without any hairs, unpunctate and glabrous; apex of anterior portion of fore tibia with sharp sickle shaped spine like projection; hind femora without any tubercles; outer portion of hind tibia with thick band of stout setae. Apex of basitibial plate simple, acute, slightly elevated from the tibia.

Metanotum: Metasomal segments are less punctate; anterior margin of T1 rounded not sharply angled.

Measurements: Given in Table 1.

Table 1: Morphometry of *Xylocopa (Biluna) nasalis* Westwood on dead bamboo plants. (N=10)

Parameters	Measurements (mm)					
	Males			Females		
	Mean ± S.D.	Min.	Max.	Mean ± S.D.	Min.	Max.
Total body length	29.00±2.08	26.6	30.31	28.36±2.83	25.10	30.01
Length of head	5.81±0.43	5.31	6.07	6.36±1.39	4.76	7.21
Width of head	7.48±0.51	6.89	7.79	7.84±1.26	6.38	8.57
Length of scape	2.45±0.24	2.18	2.64	2.65±0.52	2.06	3.00
Width of scape	0.36±0.07	0.30	0.45	0.33±0.01	0.32	0.34
Length of pedicel	0.26±0.03	0.22	0.27	0.22±0.03	0.19	0.24
Width of pedicel	0.29±0.03	0.26	0.31	0.28±0.02	0.26	0.30
Length of F1	1.11±0.13	0.96	1.19	1.16±0.27	0.85	1.32
Width of F1	0.25±0.03	0.22	0.27	0.23±0.05	0.17	0.26
Length of F2-F10 or F11	3.92±0.52	3.32	4.27	4.01±0.63	3.29	4.42
Inter antennal distance	1.03±0.23	0.77	1.19	1.16±0.13	1.02	1.26
Antenno-ocellar/ Alveolocellar distance	0.69±0.08	0.60	0.77	0.78±0.02	0.76	0.80
Anteno-occular/ Alveoocular distance	0.94±0.10	0.82	1.01	1.18±0.24	0.90	1.33
Inter - ocellar distance (b/n lateral)	0.68±0.03	0.65	0.70	0.72±0.03	0.69	0.74
Inter - ocellar distance (b/n median & lateral)	0.33±0.01	0.32	0.34	0.29±0.05	0.23	0.32
Ocello-orbital distance	0.96±0.11	0.83	1.03	1.21±0.22	0.96	1.35
Lower interoccular distance	3.52±0.33	3.15	3.75	4.02±0.66	3.26	4.41
mid inter occular distance	3.88±0.44	3.39	4.20	4.60±0.79	3.69	5.10
Upper interoccular distance	3.51±0.38	3.07	3.74	3.99±0.64	3.25	4.36
Length of mandible	2.30±0.24	2.03	2.50	2.40±0.45	1.88	2.66
Total tongue Length	9.56±2.13	7.22	11.37	7.77±0.58	7.10	8.11
Length of labial palpi	5.50±0.89	4.72	6.47	4.64±0.81	3.71	5.12

Length of clypeus	1.74±0.22	1.52	1.95	2.07±0.27	1.76	2.23
Width of clypeus	3.13±0.33	2.78	3.44	3.98±0.79	3.07	4.45
Clypeoantennal distance	0.59±0.04	0.55	0.64	0.63±0.10	0.51	0.69
clypeo-ocelllar distance	1.31±0.23	1.06	1.50	1.59±0.15	1.41	1.68
Width of eye	1.76±0.01	1.75	1.77	1.71±0.24	1.43	1.86
Length of Mesosoma	10.50±0.97	9.47	11.39	8.28±1.08	7.04	8.91
Width of Mesosoma	9.63±1.17	8.28	10.42	8.89±1.71	6.91	9.89
Length of Forewing	25.13±1.69	23.18	26.20	24.93±5.90	18.13	28.54
Length of Hindwing	15.77±1.59	13.98	17.00	15.82±3.19	12.14	17.67
Length of 1st sub marginal cell	3.69±0.60	3.00	4.10	3.44±0.85	2.46	3.94
Length of 2nd sub marginal cell	2.43±0.39	2.15	2.88	2.38±0.59	1.70	2.73
Length of 3rd sub marginal cell	4.42±1.94	2.95	6.62	3.31±0.88	2.30	3.82
Length of fore tibia	3.87±0.47	3.33	4.20	3.86±0.77	2.97	4.31
Length of fore basitarsi	3.30±0.05	3.26	3.36	3.64±0.78	2.74	4.10
Length of hind tibia	7.69±0.62	7.10	8.33	5.14±0.79	4.23	5.60
Length of hind basitarsi	5.68±0.30	5.40	6.00	6.11±2.09	3.69	7.32
Length of metasoma	12.10±0.26	11.82	12.34	12.83±1.30	11.33	13.60
Width of metasoma	11.03±1.24	9.64	12.02	10.41±2.20	7.87	11.69
I metasomal segment length	1.73±0.26	1.49	2.00	2.12±0.28	1.96	2.44
II metasomal segment length	2.80±0.31	2.44	2.99	2.64±0.40	2.18	2.87

Male (Figures 1c & 1d)

Pubescence: Integument black with dominantly black pubescence; hairs on anterior part of thorax and plurites yellowish white; fore femora without any pubescence

Head: Face with pale markings; black marginal band of the clypeus occupies its apical ¼ portion sometimes extends to apical half; supraclypeal area with pale markings, lateral pale markings reaches near to ocelli; mandible bidentate, outer teeth elongated and pointed than inner teeth, both outer and inner grooves are distinct without subapical teeth; malar area unpunctate and globrous; labrum with distinct median tubercle strongly elevated; small groove present on posterior part of median ocelli; lateral ocelli slightly elevated in position; inner margin of eyes almost parallel; face with distinct tentobrial pits; punctures on face sparse compare to abdominal tergite and posterior femora dense; tip of the last antennal segment horizontally sharp or compressed.

Mesosoma: Disc of the pronotum and fore femora unpunctate; scutum with distinct median line but parapsidal line are not clear or absent; hind trochanter not triangular; hind femur with distinct basal spine on median ridge, broad tubercle on the inner margin strong and district; outer margin of hind tibia basally unpunctate and glabrous and remaining portion covered with thick band of stout setae, apical end expanded; tegulae not elongated almost rounded, unpunctate, glabrous; wings apically with greenish golden lusture and basally with little violaceous tints; propodium with distinct median groove.

Metasoma: Punctures on metasomal tergites sparse and almost equal in all the teirgites; sternites with median elongated unpunctate band, last segment not carinated.

Genitalia (figures 1e & 1f): Genital capsule from dorsal/ventral view longer than wide, narrower at the base, gonostylus slightly slender without any projection; apex of the gonostylus with thick bunch of hairs; inner margin of gonocoxite diverging from the base; penis entirely membranous; gonocoxite without distinct ventoapical plate but weekly carinate; penis valve apically expanded; on genital capsule with strong lateral edge; base of the penis valve without any hairs.

Measurements: Given in Table 2.

Table 2: Nesting characteristics of *Xylocopa (Biluna) nasalis* Westwood on dead bamboo plants. (N=13)

Nest Characters	Measurements		
	Mean ± S.D.	**Min.**	**Max.**
Internodes length (cm)	29.83±2.32	27.00	33.00
Internodes diameter (cm)	2.03±0.16	1.80	2.30
Nest length (cm)	29.50±1.84	27.50	32.00
Internal diameter of the nest (cm)	1.44±0.25	1.20	1.90
Nest entrance hole (cm)	1.53±0.26	1.20	2.00
Number of cells/nest	5.17±2.14	3.00	8.00
Inner most cell length (1st cell) (cm)	2.17±0.21	2.00	2.50
individual cell length	2.01±0.08	1.90	2.10
Thickness of diaphragm (cm)	0.92±0.10	0.80	1.00
Pollen weight / cell (g)	1.04±0.16	0.90	1.30
Number of eggs	0.00±0.00	0.00	0.00
Number of larva	3.83±1.94	2.00	7.00
Number of female adults	1.17±0.41	1.00	2.00
Number of male adults	0.50±0.84	0.00	2.00

Fig 1: *X. nasalis* (a) Female, dorsal view (b) Female, frontal view (c) Male, dorsal view (d) Male, frontal view (e) Male genital capsule, Dorsal (f) Male genital capsule, ventral

Fig 2: Nesting habitat and architecture of of *X. nasalis* (a) Nesting habitat in the field (b) on roof of the house (c, d & e) Dissected nests of *X. nasalis* revealing the nest structure inside the bamboo culm and its residents (f) larva of *X. nasalis.*

Material Examined: India: Karnataka:3♀5♂: Bengaluru: GKVK, 12°58'N77°35'E,930m, 4.vi.14, *Prashantha, C.*6♀1♂: Belgaum: Nippani, 16°40'N74°15'E, 575m, on Pigeon Pea *Prashantha, C.*; 1♀: Chikkamagaluru: Mudigere, $13^0$07'N, $75^0$38'E, 980m04.xii.2015, *Prashantha, C.*; 2004, 1♂: V. V. Belavadi; 1♀: Niluvalligu village, 08.x.2015, Arathi pannure; Hassan: Karekere, 934m, $13^0$06'N, $76^0$10'E, 25.xii.2014, *Prashantha, C.;*17♀3♂: Chikkaballapur: Chinthamani, $12^0$23'N, $77^0$03'E, 887m, 19.i.16, *Prashantha, C;*2♀: 3.ix.13, *Prashantha, C;* 03. Xii.13, Satish; 2♀: Veeresh Kumar; 1♂:Koppal: Munirabad, $15^0$16'N, $76^0$19'E, 473m, 07.xi.15, *Najeer*; 1♀: Mysore: Chinnamballi, $12^0$05'N, $76^0$49'E, 716m, 07.xi.2015, *Prashantha, C.*; Shimoga: Navile, $13^0$58'N, $75^0$34'E, 616m, 21.ix.2015, *Prashantha, C.*

Distribution

Burma; China: Yunnan, Guangxi, Zhuang, Hunan, Sichuan, Jiangsu, Zhejiang, Fujian; India: Assam, Bangalore, Bareilley, Bombay, Calcutta, Chennai, Chittoor Cochin, Coimbatore, Cuttak, Darjeeling, Darrang, Dehradun, Nagpur, Goa, Godavari, Kotagiri, Kotdwara, Kumaon, Lucknow, Malabar, Mysore; Naga Hills; Nasik, Pankhabari, Punjab, Pusa, Chapra, Raxaul, Shillong, Sibsagar, Sikkim, Siliguri, Srinagar, Surat, Trivendrum; Java; Malaysia; Myitkyina in Upper Myanmar, Fort Stedman, Yawnghwe State, Inle Lake S. end of Taungdo, S. Shan States, Moulmein, Mandalay; Philippines, Palau & Madagascar; Sri Lanka: Habarana; Thailand.

Nesting Biology

Nests of *X.nasalis* are unbranched and constructed in pithy or hallow stem. The mass provisioned cells were separated by the which is partitions made by scrapping internal surface of the bamboo stem mixed with saliva. The nest entrances are located at the middle of the bamboo culms. A summary of nest architectural details was provided in Table 1. The average total internodes length was 29.83 cm. The average nest length was 29.50±1.84 cm. The mean internal diameter of the nest (excluding nest thickness) is 1.44±0.25 cm. The number of cells per nest ranged from 2–8 with an average cell number around 5.17±2.14 per nest. There is a difference in terms of the average individual cell lengths among cells from 13 nests. On the contrary, the innermost cell lengths were tested to be strongly different from other cells. The average number of individual adult bees found per nest ranged between 1 and 3 individuals. We found the average number of larvae 3.83±1.94 per nest; however, we did not find any nest that had eggs, pupae and all life stages of the bees present at once. The fresh weight of their unconsumed pollen masses was 1.37g (n = 4) and average weight of pollen in all the cells was 1.04±0.16.

4. Discussion

X. (*Biluna*) *nasalis* Westwood, 1838, commonly found throughout Southeast Asia. As mentioned by Hongjamrassilp and Warrit [9], it superficially resembles the sympatric species *X.* (*Mesotrichia*) *latipes* Drury, and *X.* (*M.*) *tenuiscapa* Westwood. These taxonomic comments, data on the geographical distribution and morphological measurements helps in the identification of *X. nasalis* by pollination researchers. Boontop *et al.* [13] and Hongjamrassilp and Warrit [9] provided a brief nest architectural description of *Xylocopa nasalis* nests. Here, we report detailed nest characteristics that were undescribed previously from India. The total nest length averaged at 29.50 cm, whereas the nests observed by Boontop *et al.* [13] measured 32.63 cm and that by Hongjamrassilp and Warrit [9] 25.40 cm. The sex ratio between female and male bees from this observation (4♀: 2♂ vs. 4♀: 1♂). The difference in the number of female to male bees can be explained by the collecting date, which may correspond to a later period of colony development, where most of the sister bees have emerged and stay together in the nest, whereas

male bees may have departed right after emerging from their cocoons or there is a sex ratio bias in egg-laying by the mothers as mentioned by Hongjamrassilp and Warrit[9]. Observations on such activity are needed to test these hypotheses about the skew sex ratio in the nest. The four unconsumed pollen masses have an average fresh weight of 1.37±0.13 g compared to 1.16 and 1.09 g in *X.* (*Ctenoxylocopa*) *sulcatipes* Maa, and *X.* (*Koptortosoma*) *pubescens* Spinola, species found in the desert area of the Middle East [5].

5. Conclusion

The present study is a contribution to the knowledge of the systematics and nesting architecture of *Xylocopa (Biluna) nasalis* Westwood, 1838 from India. Taxonomic comments, data on the geographical distribution and morphological measurements helps in the identification of *X. nasalis* by pollination researchers. These nesting architectural details will help to artificial trap nesting in the field and conservation of carpenter bees.

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